

Potential remediators in the rice production area of Zambales, Philippines contaminated with mine tailing

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Research Article

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Abstract

High concentration of cadmium and lead are hazardous to environment. The study isolated and identified potential fungal, bacterial and hyperaccumulating plants as bioremediators in contaminated rice ecosystem. Fungi were identified morphologically and with the use of internal transcribed spacer (ITS) region sequencing. Bacteria were identified using 16S ribosomal RNA sequences. Plants were analyzed for Cadmium and Lead accumulation in root and shoot tissues using atomic absorption spectrophotometer (AAS). Fungal species including *Penicillium janthinellum*, *Trichoderma hamatum*, *Trichoderma harzianum*, and *Curvularia lunata* along with bacterial species such as *Bacillus cereus*, *Bacillus thuringiensis*, *Pseudomonas gessardii*, *Lysinibacillus xylanilyticus*, *Lysinibacillus sphaericus*, and two species of unidentified bacteria were identified. Plants predominant in the area includes *Cyperus difformis*, *Scirpus juncooides*, *Fimbristylis miliacea*, *Centella asiatica*, *Sphagneticola trilobata*, and *Monochoria vaginalis*. Cadmium was detected in the shoots of *S. trilobata* (3.2 mg kg^{-1}) and roots of *C. asiatica* (3.6 mg kg^{-1}). Lead was found in the shoots of *C. asiatica* (2.8 mg kg^{-1}) and roots of both *S. juncooides* (15.00 mg kg^{-1}) and *F. miliacea* (15.00 mg kg^{-1}). Phytoremediation potential of *S. juncooides*, *F. miliacea*, *C. asiatica* and *S. trilobata* was observed. Heavy metal resistant microbes can be harnessed as a very useful biological tool for *in-situ* bioremediation.

Introduction

Land mining contributes not just to deforestation, erosion, sinkholes and other physical damages to land but also to significant accumulation of heavy metals and contamination the soil, ground and surface water which can lead to health issues. Releases from the mining areas, application of farm chemicals such as fertilizers and pesticides, animal defecation, sewage sludge, contaminated water used for irrigation are some of the other causes in the accumulation of heavy metals in soil. The major sink for heavy metals released into the environment ended in soil. (Khan et al. 2008; Zhang et al. 2010).

Agricultural and industrial systems done are some of the major contributors. Accumulation of waste in soil, water, and air are caused by the increasing population (Philp 2015). Controlling heavy metals using plant and microbes provides promising solutions in the reduction of the concentration and toxicity of chemical pollutants in the soil.

Soil has a natural presence of heavy metals which concentrations are increased though geologic and human activities such as mining (Alloway 1990). Storage of tailings around mine locations has been a continuing environmental problem which result to unsafe environmental pollution due to unsuccessful system in storing mine wastes (Fallagan et al. 2017). The continuous deposition of heavy metals in plant that are accumulated in rice paddy soil may lead to health disorder to rice (Shen et al. 2002). Likewise, Lone et al. (2008) described that excessive plant uptake of metals may cause acute and chronic diseases in consumers' nutrition. These conclusions have shown the danger of these mine pollutants to human health and in agreement with the statement of Jaishankar et al. (2014) on health risks associated to heavy metal that has proven to be a major threat.

Dua et al. (2002) claimed that the use of biotechnology in degrading pollutants has become one of the most rapidly developing fields of environmental restoration. Yan et al. (2020) described Phytoremediation as an eco-friendly approach as an effective and cost-effective way that could be a successful mitigation measure in revegetation of a heavy metal-polluted soil. Studies conducted on phytoremediation proved that heavy metals such as Cd, Pb, Zn, Cu, Mn, and Hg can be removed from aquatic solution (Tariq et al. 2016). Silently, the natural presence of living organisms in contaminated area has a lot of roles in degrading the toxicity of elements left in the soil or water.

Abatenh et al. (2017) described that bioremediators are mostly bacteria and fungi that has fast reproduction and growth rate in their natural habitat which consume pollutant and turning them into harmless natural compounds by turning them into harmless compounds by converting as their energy. These microorganisms were reported to have developed their mechanisms to detoxify these metals (White and Gadd 1986). Thereby, bioremediators act as toxicity neutralizers by degrading environmental pollutants (Nascimento and Xing 2006; Sardrood et al. 2013). It is then vital that these remediators be identified whether in the form of microorganism or plants in contaminated areas with mine tailing. It will provide us insights and ideas to how these areas be eradicated with unsafe pollutants and eventually recultivating with edible crops.

Phytoremediation, on the other hand, is the use of hyperaccumulating plant species for environmental remediation, which involves removing heavy metals from soils and water (Raskin et al. 1994). Hyperaccumulating plants are plant species that can absorb and accumulate metals to their tissues. Prasad and Freitas (2003) explained that trees and grasses have been used to remove and destroy hazardous contaminants from soil. Phytoremediation is a very important means in soil decontamination, water and air detoxification as well as in extraction, hyperaccumulation, and removal of heavy metals from the environment (Heinekamp and Willey 2007).

Bioremediation use by microorganisms and hyperaccumulating plant species are and environment friendly, cheap and sustainable. Plants act as storage of soil contaminants and have the natural ability to degrade heavy metals by means of various processes. Phytoremediation using hyperaccumulating plant species are significantly cheaper than conventional approaches since it is performed in naturally without any maintenance once established (Nascimento and Xing 2006) owing to plants' tolerance to heavy metal. This kind of plants' property must be exploited in cleaning-up soil contaminants. Various plants have the natural ability to degrade numerous toxic heavy metals regarded as green livers by acting as an essential sink for toxic heavy metals and compounds (Schwitzguebel 2000).

Dizon Copper Silver Mines Inc. (DCSMI), a private mining company was reported to have spilled over the Mapanuepe Lake and reached Sto. Tomas River in San Marcelino, Zambales, Philippines in 2002 (Philippine Daily Inquirer 2002). Rice and vegetable producing areas were probably affected by the mine tailings. Sixteen years after the reported contamination, a study was implemented in a two-hectare rice field near the mining site. The study was first conducted by identifying the potential fungal and bacterial remediators in the sampling area. It was continued with the addition of plant collection and identification

of possible plant remediator. This study has identified potential phytoremediators in the area in addition to the identified bioremediators.

Indigenous fungi, bacteria and predominant plants were collected from the contaminated rice paddy soils to identify potential fungal and bacterial bioremediators, and hyperaccumulating plants in the tailings-contaminated rice ecosystem. The challenge is to determine the role of these bioremediators in cleaning these heavy metals contaminated environment. Potential fungal and bacterial bioremediators and hyperaccumulating plants present in the contaminated area could be harnessed to remove or immobilize the toxic chemicals in the soil owing to their natural fast reproduction and growth rate in their natural habitat. Isolated and identified fungi, bacteria, and predominant plants present and grown in the contaminated soil with heavy metals such as cadmium and lead would determine the potential species of fungal and bacterial bioremediators, and hyperaccumulating plants phytoremediators. Elimination of environmental pollutants in the study area could be done by detecting microbes and predominant plants capable of heavy metal degradation and absorption to provide a safe food production area for farmers and consumers.

Materials And Methods

Collection of soil samples

Soil samples were collected in the rice field of Camalca, Buhawen, San Marcelino, Zambales, Philippines (14.96917°N, 120.3131°E coordinates), near the mining area on the second quarter of 2019 (Fig. 1). Soil samples were collected from nine location points and each soil sample weighs 1 kg taken at 50 cm depth. Collected soil were mixed to produce composite samples, which were placed in a clean paper bag and immediately brought to the laboratory.

Collection of plant samples

Collection of plants was undertaken in the same two-hectare rice field. Aquatic and non-aquatic plants were collected in the paddy soils of a rice field and bunds. The collected plants were brought to the laboratory and oven-dried at 70°C for 48 hours.

Representative plants were photographed and preserved for taxonomic identification. The collected plants from the area were identified, verified and authenticated by weed scientists of the Philippine Rice Research Institute. The taxonomic keys (family to species levels) are based on the book of Pancho and Obien (1995). Plant samples were separated into shoot and root portions prior to concentration analysis of heavy metals (cadmium and lead) that are accumulated in tissues.

Analysis of cadmium and lead contents of soil and plant samples

Cadmium and lead contents of soil and plants' shoot and root were analyzed using Shimadzu Analytical Methods Atomic Absorption Spectrophotometry (AAS) in the CRL Environmental Corporation-Environmental Testing Laboratory in Clark Freeport Zone, Clarkfield, Angeles City, Pampanga, Philippines.

Similar experimental procedure of AAS in determining the concentration of lead and cadmium was employed by Sisay et al. (2019) in their study. They analyzed the cadmium and lead concentration from the soil samples collected from different site of Mercato, Koch, Jimma hospital and Yetebaberut in south-western Ethiopia.

Fungal and bacterial isolation and identification

Isolation and morphological identification of fungi and bacteria were conducted in Flora and Fauna Analytical and Diagnostic Laboratory of Central Luzon State University, Philippines. Serial dilution method was used to isolate the bacteria and fungi present in the soil. Soil samples weighing 10 g were added to 100 mL of sterile distilled water. The suspension was shaken well using vortex for 30 min and properly labeled. One milliliter of suspension was transferred in a 9 mL deionized water blank using sterile pipette. The dilution was repeated three times with 1 mL of the previous suspension in a 9 mL sterile distilled water. The serial dilutions were valued as 10^{-1} through 10^{-5} . One milliliter of each of the five suspensions with 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} were spread and shaken in sterile Petri plates poured with sterile warmed melted nutrient agar (NA) for bacterial isolation. Petri plates sealed with paraffin were incubated at ambient room temperature within 18- 24 h of incubation (Fig. 2). Isolated and distinct bacterial colonies of 10^{-3} suspension were individually picked using sterile inoculating needle and were transferred aseptically into test tubes containing nutrient broth (0.1% peptone and 0.4% beef extract L-1) that served as stock culture of the bacterial isolates.

For the isolation of soil-borne fungi, 0.1 mL of each of the five suspensions with 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} were spread in sterile Petri plates. A least three drops of Streptomycin sulfate using sterile syringe were added. Warmed sterile melted potato dextrose agar (PDA) was poured in sterile petri plates and then properly labeled. The Petri plates were incubated at ambient room temperature to allow the growth of fungal colonies within three to five days of incubation (Fig. 3). Isolated and distinct fungal colonies were individually picked using sterile inoculating needle and transferred aseptically into test tubes containing slanted PDA. These tubes served as stock cultures of the fungal isolates.

DNA extraction and PCR product for molecular identification of fungi and bacteria isolates was conducted in Tuklas - Lunas Center of the university's Department of Biological Sciences in the Science City of Muñoz, Nueva Ecija, Philippines.

Morphological identification of fungal isolates

Colony morphology was used to describe the characteristics of an individual colony of fungus growing on agar in a Petri dish. It helped in the identification of the type and kind of the organism. Soil-borne fungi were observed in agar block culture known as slide culture technique. In this method, development and structures were observed without being disturbed or constantly moved when the tissues were lifted from the glass slide.

In agar block culture, a bent aluminum foil rod on moist tissue paper held a glass slide in a sterilized Petri plate. An agar block was then cut aseptically from agar plate culture, lifted and placed on the surface of the slide. Mycelium or spores of desired organism were inoculated in the four sides of the agar block. A cover slide was centrally placed upon the inoculated agar block. The Petri dish was covered and incubated until the desired growth was obtained. Sterilized water was added to the tissue paper maintain the moisture ambient during incubation.

Cover slip was carefully lifted and placed on a drop of lactophenol on a clean slide at the sight of mycelial growth. Another drop of lactophenol was placed on the center of the fungal growth then covered with a new clean cover slip. Mounted glass slides were prepared for microscopic examination for the morphological identification of the fungal isolates in accordance with protocols used by Quimio and Hanlin (1999) and Watanabe (2010).

Polymerase Chain Reaction (PCR) for the molecular identification of fungal and bacterial isolates

Fungal isolates with uncertain identity thru morphology were subjected to molecular identification using internal transcript spacer (ITS) region sequencing. In DNA extraction of fungal isolates, the mycelia were mashed with 1x CTAB buffer (20 g of CTAB dissolved in 860 mL sterile double distilled water, 81.82 g NaCl, 100 mL 1M Tris with pH 8.0, 40 mL 0.5M EDTA with pH 8.1) until they become sticky. The sticky samples were then transferred to a 1.5 mL tube and incubated for 3 hours. Fifty microliters (μ L) of 20% SDS was added to samples and mixed using vortex before incubation at 65°C for 30 minutes to one hour using dry bath (Labnet D1200- 230V Accublock Digital dry bath). After incubation and cooling, 750 μ L of chloroform was added to samples, mixed thoroughly using vortex. Tubes containing the suspension were centrifuged for 30 minutes at 10,000 rpm. Upper layer of the solution was transferred to a new 1.5 mL sterile tube. Subsequently, 600 μ L of ice-cold isopropanol was added and incubated overnight at -20°C. Samples were mixed gently then centrifuge for 10 minutes at 10,000 rpm after incubation. The isopropanol was decanted and the pellet was washed with 500 μ L of 70% ethanol. The samples were subjected to centrifuge for 10,000 rpm for 3 minutes. The ethanol was decanted and the formed pellets were air-dried until the alcohol was completely removed from the pellet. The pellet was dissolved using TE buffer with RNase. The extracted DNA was stored in 4°C until usage. The final component concentration per 25 μ L of sample cocktail included 1.0 μ L of DNA extract, 2.5 μ L 10x PCR Buffer (KAPA), 1.0 μ L DNTP Mix (KAPA), 1.5 μ L MgCl₂, 2.0 μ L ITS, mixed 1Forward (5'-CTT GGT CAT TTA GAG GAAGTA A-3') primer and 4Reverse (5'-TCC TCC GCT TAT GC-3') primer (Bruns and Gardes 1993), 16.9 μ L sterilized distilled water, and 0.1 Taq Polymerase (KAPA). Gene amplification of the PCR samples involved up to 35 cycles with initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing at 51.1°C for 30 seconds, extension at 72°C for 1 minute, final extension at 72°C for 10 minutes and final hold at 4°C.

Metagenomic sequencing of bacterial samples has become the gold standard for profiling microbial populations, but 16S rRNA profiling remains widely used due to advantages in sample throughput, cost, and sensitivity even though the approach is hampered by primer bias and lack of specificity (Schriefer et

al. 2018). In this respect, similar approach applicable to bacterial identification was used in this research.

Bacterial isolates in nutrient agar (NA) plates were identified by subjecting to colony polymerase chain reaction (PCR) method using 16S ribosomal RNA gene sequencing. Single colony of each bacterial isolate was placed in tube before the dilution of final component. The final component concentration per 25 µl were 1.0 µl of DNA extract, 2.5 µl 10x PCR Buffer (KAPA), 1.0 µl DNTP Mix (KAPA), 1.5 µl MgCl, 1.0 µl 27Forward (5'-AGA GTT TGA TCM TGG CTC AG-3') primer (Lane, 1991), 1.0 µl 1492Reverse (5'-CGG TTA CCT TGT TAC GAC TT-3') primer (Jiang et al. 2006), 17.9 µl sterilized distilled water and 0.1 Taq Polymerase (KAPA). The PCR profile that was used to amplify the gene was made up to 35 cycles with initial denaturation at 95°C for 2 minutes, denaturation at 95°C for 20 seconds, annealing at 54°C, 56°C and 56.5°C for 30 seconds, extension at 72°C for 1-minute, final extension at 72°C for 5 min and final hold at 4°C.

One (1) µl of amplification products and the 1kb DNA ladder stained with 1 µl gel red (Biotium) was run for 30 minutes at 100 V on 1.0% agarose gel (prepared in 1x TAE) and analyzed under gel photo documentation system (Labnet GDS- 1302 Enduro Imaging System).

Sequence analyses of fungal and bacterial isolates

Fungal and bacterial PCR products were sent to Apical Scientific Sequencing in Malaysia for PCR purification and sequencing after confirmation of the expected size of amplified fragments. The generated chromatogram was evaluated using four peaks (NUCLEOBYTES.COM) software in the identification of fungal and bacterial species.

Occurrence of fungal and bacterial colonies

Percent fungal occurrence was resolved by percent occurrence formula, while bacteria present in the soil were determined by presence and absence only. Data observed included the total accumulation of microbial colony and the soil concentration of heavy metals.

Percent (%) Occurrence Formula:

$$\text{Percent (\%) Occurrence} = \frac{\text{No. of colonies of species per plate}}{\text{Total fungal population}} \times 100$$

Results

Fungal Species Isolated from the Soil.

Classification used in fungal identification was based on the book of Watanabe (2010), that details the morphology of cultured fungi and key to species. Fungal specimens were viewed under the microscope and compared with the morphology of cultured fungi in the book. Fungal with unclear and doubtful characteristics under microscope was molecularly identified using ITS region sequence.

Three species of fungi were identified in accordance to morphologies of cultured fungi and key to species of Watanabe (2010) and one specie was molecularly identified using ITS region sequence. *P.*

janthinellum, *T. hamatum* and *C. lunata* fungal isolates were identified according to their morphology and cultural characteristics. On the other hand, *T. harzianum* strain (ACCC32889) was molecularly identified because of unconvinced morphological characteristics. The following are the cultural and morphological characteristics of the fungal isolates:

Penicillium janthinellum

This organism belongs to Class *Eurotiomycetes*, Order *Eurotiales* and Family *Trichomaceae* based from the observed color of different colonies grown in Potato Dextrose Agar that are velvety, pale grayish green in the obverse, and pale yellowish brown in reverse. They grow at a rate of 10 to 20 mm in 5 days of inoculation (DAI) under room temperature atmosphere (Figs. 4A and B).

Conidiophores of this organism were found to have an erect transparent or nearly transparent appearance (hyaline) that resembles like a small clump of hair (penicillate), branched at the apexes with a hair or flower like structure (verticillate) and a spiral shape medulla with terminal phialides. It has conidia that are formed in a row (catenulate) arranged in a ring-like series on each phialide. They form contrasting conidial heads, phialides pen pointed with tips that narrows at one end. Conidia are formed on the tip of the phialide (phialosporous) with pale green color but dark in mass, oval-shaped (ellipsoidal) or having the shape of a sphere (sub globose). It is a single celled, smooth and ending abruptly in a small distinct point (apiculate) at one end (Fig. 4C).

Trichoderma hamatum

This organism belongs to Class *Eurotiomycetes*, Order *Eurotiales* and Family *Trichomaceae*. The color of colonies in the PDA were found to be initially white at the surrounding and turning greenish to the inside. It also forms cushions distributed in the obverse and pale yellowish brown in reverse. This microorganism has a growth rate of 10 to 20 mm in 5 DAI at room temperature environment (Figs. 5A and B).

Conidiophores are developed on cushion structure referred to as a pillow-shaped due to its shapes with rounded edges. It has an erect transparent appearance, branched, bearing spore masses on alternate resembling a flower arrangement phialides together with setae-like sterile hyphae, an elongated threadlike element of the mycelium. The organism has a densely arranged, short and thick phialides. Hyphae are curved bristle like part, gradually tapering toward apex and septate. Conidia are phialosporous, hyaline, ellipsoidal and single-celled ovate. The color of granulated chlamydospore is pale brown having an ellipsoidal shape (Fig. 5C).

Trichoderma harzianum

This organism belongs to Class *Eurotiomycetes*, Order *Eurotiales* and Family *Trichomaceae*. The shape of the observed colonies on PDA are needle-like with a white color produced at the front and cottony white in reverse side. This fungus was molecularly identified using ITS region sequence due to unclear and doubtful characteristics under microscope (Figs. 6A and B).

The structure of conidiospore is hyaline, erect, branched, bearing spore masses apically at verticillate phialides with short and thick phialides. Single-celled conidia are phialosporous and hyaline with a globose, sub-globose, ovate shape. The color of chlamydospore is brown and the shape is sub-globose (Fig. 6C).

Curvularia lunata

This fungus belongs to Class *Euscomycetes*, Order *Pleosporales* and Family *Pleosporaceae*. The observed color of colonies on PDA are brown to grey in color in obverse, tinted brown in color in reverse (Figs. 7A and B).

The observed structure of conidiophores is erect and its color is brown. Its structure could be simple or branched, some are straight and some are curved. It bears conidia apically and laterally and easily noticed pores are left after detachment of conidia. Conidia are produced by an extension of the inner wall of the conidiogenous cell and extrusion through a pore in the wall of the conidiophore. It is egg-shaped that are mostly 4-celled and darker brown color in two central cells that are curved and larger in the penultimate cells with indistinct scar on a spore at the point of attachment basally (hilum) (Fig. 7C).

Percent occurrence of Fungi in the Soil

Percent occurrence was used to measure the number of times the organism occurs in the area. In this study, two species of *Trichoderma*, *T. hamatum* and *T. harzianum*, were found in the soil contaminated with mine tailings. Among the four isolated fungi, *T. hamatum* registered the highest occurrence with 52.78% while the lowest frequency was exhibited by *C. lunata* with a value of 5.56% (Table 1).

The occurrence of various species of *Penicillium* is common in soils, foods, drinks and even in indoor air and this microorganism can also colonize soils contaminated with heavy metals (Banker et al. 1997). Based from the findings of Knudtson and Kirkbride (1992), most species of *Curvularia* are facultative pathogens of soil, plants, and cereals in tropical or subtropical areas, while the remaining few are found in temperate zones.

Bacterial Species Isolated from Soil

Bacterial biosorption bioremediation is essentially used for the removal of pollutants in waters like metals and dyes that are non-biodegradable pollutants (Vijayaraghavan and Yun 2008). The effective way to screen and collect samples is in a big scale. It is a cumbersome activity but one of the effective ways of remediating the contaminated soil with mine tailings. Bacteria have developed resistance mechanism

and system in heavy metal consumption thereby detoxifying pollutants mostly for their survival (Mustapha and Halimoon 2015).

Identities of ten out of twelve bacteria isolated from the soil were molecularly recognized using 16S ribosomal RNA gene sequencing. The DNA amplification of the two isolates were poor and confirmation was not possible (Fig. 8). The identified ten bacteria included five accessions of *B. cereus*, two accessions of *B. thuringiensis*, *P. gessardii*, *L. xylanilyticus* and *L. sphaericus* (Table 2).

Potentiality of fungi and bacteria as bioremediation agents

It is assumed that the ability of a certain fungi and bacteria to be able to live in an environment contaminated with heavy metals make them potential pollutant feeders. Their resistance in the soil with the presence of heavy metals has a high probability that they consume pollutants as their source of energy for their continuous existence. Microorganisms can be used in remediation of the contaminated environment as explained by Abatenh et al. (2017). According to Jaiswal (2011) isolated fungi from contaminated soils with mine tailings have the potential in reducing, mobilizing or immobilizing heavy metals through different processes like complexation, biomethylation, sorption, and oxidation- reduction process. Siddiquee et al. (2015) reported that fungal amendments using different *Trichoderma* strains were effective in the mobilizing, chelating and extracting chromium, cadmium, copper, nickel and zinc independently of the plant species used. Mustapha and Halimoon (2015) reported that *P. simplicium* has the ability to absorb high cadmium while *P. chrysogenum* has the ability to absorb high amount of lead. They also reported that species of *Penicillium* and *Trichoderma* were utilized as bio sorbent materials while *C. lunata* cannot be used in bioremediation because it can cause plant disease.

The species of bacteria isolated from the study site in Zambales, Philippines have been reported to remove pollutants from contaminated environment. Sriram et al. (2011) reported that *B. cereus* is heavy metal resistant and can also be harnessed as a very useful biological tool for *in situ* bioremediation. Oves et al. (2012) reported that *B. thuringiensis* strain OSM29 showed a clear metal eliminating potential in the presence of the varying concentrations of **heavy metals** (25-150 mg kg⁻¹) such as **chromium**, **cadmium**, **lead**, copper and nickel. They noticed a rapid biosorption of each metal which could be useful in a large-scale environmental cleansing of contaminated areas.

An isolated *P. gessardii* strain LZ-E from a petrochemical wastewater discharge was found out by Huang et al. (2016) to degrade naphthalene and reduces hexavalent chromium [Cr(VI)], simultaneously. They further stated that with eight batches in 16 days, this microorganism continuously remediates naphthalene and Cr(VI) at rates of 15 mg L⁻¹ h⁻¹ and 0.20 mg L⁻¹ h⁻¹ of 800 mg L⁻¹ naphthalene and 10 mg L⁻¹ Cr(VI). Cr(VI) compounds are known to be toxic as well as carcinogenic (Costa, 1997).

A native Colombian strain of *L. sphaericus* OT4b.31 was found out to be useful not only in bioremediation of heavy metals such as cadmium, zinc, cobalt, copper, nickel, chromium and arsenic but also effective as biological control of agricultural pests (Peña-Montenegro and Dussan 2013).

Furthermore, Rahman et al. (2015) reported that *L. sphaericus* is a significant bacterium in eradicating arsenics and other toxic metals from the contaminated sources.

Based on the results of this study, the fungal and bacterial isolates are potential bioremediators because of their ability to grow in contaminated soil with mine tailings in accordance to what was reported by Chen et al. (2003). Microorganisms have developed mechanisms to resist the toxic effects since heavy metals are abundant in the environment (White and Gadd 1986). Numerous microorganisms are capable of thriving in soil with presence of high concentration of heavy metals (Anderson and Cook 2004).

Soil Properties and Heavy Metals Concentration

It was found out that the sampled soil collected in the area is a silt loam type. Soil samples also contained not more than 70% combination of silt and clay and minimum 2% of its composition is sand. The pH of soil ranged from 4.26 to 4.27 which classified to be strongly acidic. The cadmium content detected is 0.50 mg kg^{-1} and the lead content is 17.00 mg kg^{-1} which are lower than the recommended upper limit for the concentration of heavy metals in soil. The maximal permissible concentration in the soil is 0.76 mg kg^{-1} for cadmium and 55.00 mg kg^{-1} for lead (Crommentuijn et al. 1997).

Plants Present in the Area

Identification of plants present in the area was done using the manual of rice field weeds in the Philippines (Pancho and Obien 1995). While the sampled area is planted with rice, plants of different species are also present. The continuous production of rice for human consumption in the contaminated soil had led to the necessity of observing bioremediators including plants. It was observed that several plants are present in the area which are commonly weeds of rice. From the collected plant samples, six species are predominantly growing in the study sites, namely: *Cyperus difformis* L., *Scirpus juncooides* Roxb., *Fimbristylis miliacea* (L.) Vahl., *Centella asiatica* (L.) Urban, *Sphagneticola trilobata* (L.) Pruski, and *Monochoria vaginalis* (Burm.f.) C. Presl. These plants usually grow in rice fields across the country. The distinguishing characteristics of the collected plants are described below:

Cyperus difformis

The common names of this plant in Tagalog language are payong-payong and gumi while it is called siraw-siraw and balayang in Ilocano, one of the several dialects in the Philippines. This plant was found growing in both irrigated lowland and rainfed area. It can grow as tall as 75 cm. It has stems that are pale green and sharply three-angled at the top, has shorter leaves than stems, umbellate inflorescence that is simple or compound and numerous spikelet that are globose (Figure 9A).

Scirpus juncooides

The common names of this plant in Tagalog language are bitubituinan and balbas-kalabaw. It is called apulid in Ilocano dialect. *Scirpus juncooides* is found in irrigated lowland and marshland areas. The stems

are erect and slender. Leaves degenerate to become sheath-like and cover the base of the stems. Fruits are brown to black when mature and broadly elliptical (Figure 9B).

Fimbristylis miliacea

The common names of this plant are agor, buntot pusa and Taulat in Tagalog language. It is called gumi in Pangasinanese dialect and siraw- siraw in Ilocano dialect. Usually, this plant is found in both irrigated lowland and rainfed areas. It is erect with flattened stems that bears two to four unequal bracts that are shorter than the inflorescence. Leaves are linear, flat, soft and overlapping in two rows. Spikelet were numerous, globose to ovoid in shape, and brown to brown-orange (Figure 9C).

Centella asiatica

The common names of this plant are takip kuhol, takip-suso and tapigan-daga in Tagalog language. *Centella asiatica* thrives in irrigated lowland mostly found on lower part of the bunds of rice paddy. Leaves are rounded to reniform, crenate-dentate, 2 to 5 cm in diameter and its petioles measures from 1 to 5 cm or even longer. Peduncles are in pairs of three, less than 1 cm long, each with usually three-sessile flowers at the apex that is enclosed by a pair of ovate bracts. Petals are dark purple, ovate and about 1mm long. Carpels are cylindrical-compressed, about 2.5 mm long, white or green, reticulate, each with nine sub similar longitudinal ridges (Figure 9D).

Sphagneticola trilobata

The common names of this plant are yellow dots, wendelia and trailing daisy in English language. No names in other language or dialects were gathered. This weed occurs mostly on the bunds of rice field in both irrigated lowland and rainfed areas. It had mat-forming perennial herb up to 30 cm height and a rounded stems up to 40 cm long. Rooting at nodes with the flowering stems ascending. Leaves are fleshy and hairy with 4 to 9 cm long and 2 to 5 cm wide. Flowers are 2 cm across and golden yellow (Figure 9E).

Monochoria vaginalis

Its common names are gabi-gabihan and gabing-uwak in Tagalog language while it is called lapalapa and lil-lagut in Ilocano dialect. It is called gabi-gabi in Visayan dialect. *Monochoria vaginalis* is commonly found in irrigated lowland areas. It is one of the most invasive plants in the rice fields. Distinguishing characteristics are fleshy, semi-aquatic monocotyledon weed that has shiny appearance in the field. Its stems are soft, erect and rooting at the nodes. Flower stalks are long bearing lilac-blue or violet petals that are arranged in two to six groups. Its leaves are heart-shaped and petioles that are soft and hollow (Figure 9F).

Cadmium and lead contents in plant tissues

Cadmium and lead accumulation in shoot (above ground), and root parts were assessed to determine the ability of these parts to accumulate the heavy metals. The concentration of cadmium in the roots and

shoots of different plant species is presented in Table 3. Among the different plant species, *S. trilobata* exhibited the highest cadmium accumulation in shoot with 3.10 mg kg^{-1} while cadmium was not detected in *M. vaginalis*. Meanwhile, the highest cadmium accumulation in root parts was observed in *C. asiatica* with 3.60 mg kg^{-1} while cadmium was not observed in the roots of *C. difformis*. The results of the present study suggest that *S. juncooides*, *F. miliacea*, *C. asiatica*, *S. trilobata* and *M. vaginalis* collected have the ability to absorb cadmium from the soil as indicated by the presence of heavy metals in the roots. On the other hand, *C. difformis*, *S. juncooides*, *F. milicea*, *C. asiatica* and *S. trilobata* translocated cadmium to the shoots.

The concentration of lead in the shoots and roots of different plants species is also presented in Table 3. It can be noted that among the different plant species analyzed, *Centella asiatica* is the only plant species that contain lead with 2.80 mg kg^{-1} in shoots. On the other hand, *S. juncooides* and *F. miliacea* registered the highest lead accumulation in the root part with 15.00 mg kg^{-1} while this was not detected in *C. difformis*, *C. asiatica*, and *S. trilobata* roots.

Several plants species have been previously reported to accumulate different heavy metals. For instance, Ewais (1997) determined the effects of cadmium, nickel and lead on the growth of potted weed plants *C. difformis*, *Chenopodium ambrosioides* and *Digitaria sanguinalis*. He found that three heavy metals inhibited the shoot growth but were less suppressive to root growth. He noted that addition of the heavy metals in the soil resulted to the increase of their concentration in both roots and shoots. The roots contain higher concentration than the shoots. Rotkittihun et al. (2006) reported that *C. difformis* can uptake lead. However, this weed is not classified as hyperaccumulating plant because of the low uptake of lead as compared to lead concentration of the soil.

Gaspar (2013) disclosed that cadmium and lead in soil greater than 25 mg/kg are toxic to shoots, roots and growth of *F. miliacea*, respectively. He recognized this species as hyperaccumulating plant which implies that it could absorb greater concentration of heavy metals. This plant species is classified as phytostabilizer of heavy metal in terms of bioaccumulation factors (BAF). *F. mileacea* was classified as effective translocators of cadmium and lead lower than 25 mg kg^{-1} concentration in soil.

In another study, Mokhtar et al. (2011) reported that iron absorption was the highest followed by zinc, lead, copper, nickel and cadmium in roots and shoots of *C. asiatica*. They discovered that for all metal accumulations, roots displayed the highest level followed by leaves and stems.

On the other hand, Ong et al. (2011) found out that the metal uptake capacity of *C. asiatica* differ in each part of the plant. Roots absorption is higher than in stem and leaves have the least. It was claimed that *C. asiatica* has the potential of being used as a plant monitor for heavy metal pollution since a relationship was observed between the concentrations of heavy metals in different parts of the plant and the metal levels in the most contaminated soil.

Bert et al. (2003) as cited by Hakeem et al. (2015) disclosed that heavy metals are generally translocated in cell vacuoles which is done through enhanced tonoplast, a membrane which bounds the chief vacuole of the plant cell. The translocation process to cell vacuoles is reported to be controlled by gene. The finding was in connection with the account of De (2000) which clarifies that large number of heavy metals including cadmium and lead are deposited in vacuoles. Yoon et al. (2006) detailed that translocation in higher level is suitable for use in phytoextraction of the shoot part and phytostabilization on the root part.

Discussion

Mining is one of the important and critical profitable businesses in the country that supports the development of a nation. However, it affects the environment by contaminating the agricultural soil with heavy metals where most of food are produced. Agriculture is far more important than mining because it feeds the nation. The use of biological processes for the cleanup of contaminated soil, though a time taking process is considered vital. Plant and soil microorganisms play an important role in the recovery of degraded rice lands and have important influence on soil fertility and plant health.

Plant and microbial usefulness to reduce a wide array of contaminants makes bioremediation technologies could be applied in different soil environments. The United States Environmental Protection Agency (2001) however reported that bioremediation is still not a common practice despite numerous research findings being inexpensive and capable of removing contaminants produced by land mining. This study had isolated and identified fungi, bacteria and plants that could possibly remove or reduce contaminants present in the rice field as possible candidates for bioremediation and phytoremediation. Bioremediation systems are still limited by additional findings on the capabilities of the native microbes to tolerate concentration of heavy metals. Cadmium and lead collected in the shoot and root parts of the plants were determined using atomic absorption spectrophotometer (AAS).

In this study, four fungal species namely *P. janthinellum*, *T. hamatum*, *T. harzianum*, and *C. lunata* and five bacterial species such as *B. cereus*, *B. thuringiensis*, *P. gessardii*, *L. xylanilyticus*, and *L. sphaericus* were isolated from soil samples taken in the contaminated agricultural area of Zambales, Philippines. Only *T. harzianum* was identified using molecular approach due to its unclear and doubtful characteristics under microscope. ITS coding regions have an important role in the development of functional rRNA with sequence variations among species showing promise as unique regions for molecular analyses (Iwen et al. 2002). They described that the intervening internal transcribed spacer (ITS) regions have great possibility as targets in molecular analyses for the classification and identification of fungi. Owing to numerous usages of the ITS region in the identification of microorganism, we can conclude that this is the most widely sequenced DNA region in molecular ecology of fungi. It has been recommended as the universal DNA barcode marker sequence for fungi (Schoch et al. 2012).

A follow up study that will examine the two unidentified bacteria on their identity and their ability to eliminate heavy metal is needed. The ability of the isolated bacteria and fungi to absorb heavy metals should be evaluated by growing them in a medium contaminated with lead and cadmium to determine tolerance. Level of naturally occurring microbial population in the test area in relation to their level of contaminant reduction can also be considered in future studies following the general strategy discussed in the book, *In Situ Bioremediation* (National Research Council 1993). The book discussed that there should be evidences like loss of contaminants, assays showing that microorganisms have the potential to alter contaminants and biodegradation potential is actually realized. It also emphasized that bioremediation should be happening as a proof that contaminant concentrations have decreased and that microbes caused the decrease.

The concentration of heavy metals in fungi and bacterial cells is also recommended for further investigation following the bioaccumulation procedure described by Liaquat et al. (2020). They used metal quantification using atomic absorption spectrophotometer with a graphite furnace. The phytoremediation potential of *S. juncooides*, *F. miliacea*, *C. asiatica* and *S. trilobata* should be further evaluated. Tolerance exhibited by these fungi to heavy metals may show their abilities to undertake heavy metal clean up. Their identification in the sampling area generates an awareness that fungal bioremediation in the area may reduce the concentration of heavy metals.

C. difformis, *S. juncooides*, *F. miliacea*, *C. asiatica*, *S. trilobata*, and *M. vaginalis* are the six plant species that are predominantly present in rice ecosystem contaminated with mine tailings. Cadmium was found present in the shoots and roots of *S. juncooides*, *F. miliacea*, *C. asiatica* and *S. trilobata* while absent in the roots of *C. difformis* and shoots of *M. vaginalis*. On the other hand, lead was detected in the shoots of *C. asiatica* and roots of *S. juncooides* and *F. miliacea*. Hence, these plant species are potential phytoremediation agents.

Mine tailings particularly cadmium and lead contaminated the rice field area as found in plant tissues. To avoid alteration of soil by the use of costly physical and chemical technologies in the removal of heavy metals, the activities of fungi, bacteria and plant species could be considered as alternative and a no cost natural soil remediation. Bioremediation has been generally accepted as an inexpensive method to reestablish biological activity and physical structure of soil (Lai et al. 2016). Their claim that lead and zinc are among the most common contaminants in soils originated by mineral exploitation is in agreement with the lead that was found in tissues of the collected plants. A study to identify which of the remediators is the most ideal, or which is the best in group may provide a broader outlook in soil remediation. A more focus identification of plant species that are innate in the area which authenticates a better tolerance to local situations in contrast to introduced or invasive species in order to reduce injury to human, animal and plants which pose a great danger on the ecosystem must be done.

Declarations

Ethical Approval

The research does not contain any studies with human and animal participants performed by any of the authors. Authors declare that they have no conflict of interest and all procedures performed in the study were in accordance with ethical standards.

Consent to Participate

We have met a consensus to participate in the research conducted following what has been transcribed during the study conceptualization. Our participation to conduct the research reflected in the manuscript is completely voluntary. We understand that we are free to contact any of the individuals involved in the research to seek further clarification and information.

Consent to Publish

We voluntarily give our consent for the publication of identifiable details, which includes photographs to be published in Environmental Science and Pollution Research. We fully understand that our partaking is voluntary and aware that all Springer journals may be available in both print and online, and will be available to public consumption.

Owing to these, we completely understand that anyone can read material published in this Journal. I understand that readers may include professionals in different fields, scholarly researchers as well as journalists and the general public.

Author Contributions

Both authors contributed from the conceptualization of the research to the conduct, data analysis and manuscript writing. The first draft of the manuscript was written by Perfecto Salvador Ramos and Oliver Escaño Manangkil provided references and improved the document. Both authors have read and approved the final manuscript.

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Competing Interests

Both authors declare they are currently employed with the Philippine Department of Agriculture, Philippine Rice Research Institute during the submission of the manuscript. Perfecto Ramos, Jr. was on a study leave during the conduct of the study.

Availability of data and materials

All data produced and examined during the conduct of the study are included in this manuscript. The raw datasets gathered and analyzed are available and being kept by Perfecto S. Ramos.

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References

1. Abatenh E, Gizaw B, Tsegaye Z, Wassie M (2017) The role of microorganisms in bioremediation - A review. *Open J Environ Bio* 2(1):030–046
2. Alloway BJ (1990) Heavy metal in soils. John Wiley and Sons, New York. NY. USA
3. Anderson CR, Cook GM (2004) Isolation and characterization of arsenate-reducing bacteria from arsenic contaminated sites in New Zealand. *Curr Microbiol* 48:341–347
4. Banker S, Frisvad JC, Rosendahl S (1997) Taxonomy of *Penicillium chrysogenum* and related xerophilic species, based on isozyme analysis. *Mycol Res* 101:617–624
5. Bert V, Meerts P, Saumitou- Laprade P, Salis P, Gruber W, Verbruggen N (2003) Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. *Plant Soil* 249:9–18
6. Bruns TD, Gardes M (1993) Molecular tools for the identification of ectomycorrhizal fungi-taxa-specific oligonucleotide probes for suilloid fungi. *Mol Eco* 2(4):233–242
7. Chen G, Zhu H, Zhang Y (2003) Soil activities and carbon and nitrogen fixation. *Res Microbiol* 154:393–398
8. Costa M (1997) Toxicity and carcinogenicity of Cr(VI) in animal models and humans. *Crit Rev Toxicol* 27(5):431–442. doi: 10.3109/10408449709078442
9. Crommentuijn T, Struijs J, Van de Meent D, Peijnenburg WJGM, Van den Hoop MAGT (1997) Added risk approach to derive maximum permissible concentrations for heavy metals: how to take natural background levels into account. *Ecotoxicol Environ Saf* 37(2):112–118
10. De D (2000) Plant Cells Vacuoles: An introduction. Csiro Publishing. Collingwood, Australia. DOI: 10.1071/9780643090064
11. Dua M, Singh A, Sethunathan N, Johri A (2002) Biotechnology and bioremediation: successes and limitations. *Appl Microbiol Biotechnol* 59:143–152
12. Ewais EA (1997) Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. *Biol Plant* 39(3):403–410
13. Falagan C, Grail BM, Johnson DB (2017) New approaches for extracting and recovering metals from mine tailings *Miner Eng.* 71-78
14. Hakeem KR, Sabir M, Ozturk M, Mermut A (2015) Soil remediation and plants: prospects and challenges. Academic Press, p 707

15. Heinekamp Y, Willey N (2007) Using real-time polymerase chain reaction to quantify gene expression in plants exposed to radioactivity.. In: In: Willey N (ed) *Phytoremediation: Methods and reviews*. Humana Press, Totowa, pp 59–70
16. Huang H, Wu K, Khan A, Jiang Y, Ling Z, Liu P, Chen Y, Tao X, Li X (2016) A novel *Pseudomonas gessardii* strain LZ-E simultaneously degrades naphthalene and reduces hexavalent chromium. *Bioresour Technol* 370–378. <https://doi.org/10.1016/j.biortech.2016.02.015>
17. Iwen PC, Hinrichs SH, Rupp ME (2002) Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human fungal pathogens. *Med Mycol* 40(1):87–109. doi: 10.1080/mmy.40.1.87.109
18. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol*. 2014 Jun; 7(2): 60–72. doi: 10.2478/intox-2014-0009
19. Jaiswal S (2011) Role of rhizobacteria in reduction of arsenic uptake by plants: A review. *J Bioremed Biodegrad* 2(126). <http://dx.doi.org/10.4172/2155-6199.1000126>
20. Jiang H, Dong H, Zhang G, Yu B, Chapman LR, Fields MW (2006) Microbial diversity in water and sediment of Lake Chaka, an athalassohaline lake in northwestern China. *Appl Environ Microbiol* 72(6):3832–3845. <https://doi.org/10.1128/AEM.02869-05>
21. Iwen PS, Hinrichs SH, Rupp ME (2002) Utilization of the Internal Transcribed Spacer regions as molecular targets to detect and identify human fungal pathogens. *Med Mycol* 40(1):87–109. doi: 10.1080/mmy.40.1.87.109
22. Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG (2008) Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ Pollut* 152(3):686–692
23. Knudtson WU, Kirkbride CA (1992) Fungi associated with bovine abortion in the northern plains states (USA). *J Vet Diagn Invest* 4(2):18–185
24. Lai T, Cappai G, Carucci A (2016) Phytoremediation of mining areas: An overview of application in lead- and zinc-contaminated soils.. In: In: Ansari A, Gill S, Gill R, Lanza G, Newman L (eds) *Phytoremediation*. Springer, Cham. https://doi.org/10.1007/978-3-319-40148-5_1
25. Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. John Wiley and Sons, New York, NY, pp 115–175
26. Liaquat F, Munis MFH, Haroon U, Arif S, Saqib S, Zaman W, Khan AR, Shi J, Che S, Liu Q (2020) Evaluation of metal tolerance of fungal strains isolated from contaminated mining soil of Nanjing. *China Biology* 9:469. doi:10.3390/biology9120469
27. Lone MI, He ZL, Stoffella PJ, Yang XE (2008) Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. *J Zhejiang Univ Sci B* 9(3):210–220. doi: 10.1631/jzus.B0710633
28. Mokhtar H, Morad N, Fizri FFA (2011) Phytoaccumulation of copper from aqueous solutions using *Eichhornia crassipes* and *Centella asiatica*. *Int J Environ Sci Dev* 2(3):205

29. Mustapha MU, Halimoon N (2015) Microorganisms and biosorption of heavy metals in the environment: A Review Paper. *J Microb Biochem Technol* 7:5
30. National Research Council (1993) *In Situ Bioremediation: When does it work?* The National Academies Press, Washington, DC, pp 63–64. <https://doi.org/10.17226/2131>
31. Nascimento CWA, Xing B (2006) Phytoextraction: A review on enhanced metal availability and plant accumulation. *Sci Agric*(63):299–311
32. Ong GH, Yap CK, Maziah M, Tan SG (2011) Heavy metal accumulation in a medical plant *Centella asiatica* from Peninsular Malaysia. *J Biol Sci* 11(2):146–155
33. Oves M, Khan MS, Zaidi A (2013) Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil. *Saudi Journal of Biological Sciences* 20(2):121–129
34. Pancho JV, Obien SR (1995) *Manual of ricefield weeds in the Philippines*. Philippine Rice Research Institute, Maligaya, Science City Muñoz, Nueva Ecija. Philippines. 543
35. Peña-Montenegro TD, Dussán J (2013) The genomic standards consortium genome sequence and description of the heavy metal tolerant bacterium *Lysinibacillus sphaericus* strain OT4b.31. *Standards. in Genomic Sciences* 9:4256. DOI:10.4056/sigs.4227894
36. Philippine Daily Inquirer (2002) The San Marcelino tailings dam spill (Zambales, Philippines). (Accessed September 12, 2002)
37. Philp R (2015) *Bioremediation: The pollution solution?* Microbiology Society. <https://microbiologysociety.org/blog/bioremediation-the-pollution-solution.html>
38. Prasad MNV, Freitas HMD (2003) Metal hyperaccumulation in plants - biodiversity prospecting for phytoremediation technology. *Electron J Biotechn* 6:285–321
39. Quimio T, Hanlin RT (1999) *Illustrated genera and species of plant pathogenic fungi in the tropics*. University of the Philippines Los Baños
40. Raskin IP, Nanda Kumar BA, Dushenkov S (1994) Phytoremediation - using plants to clean up soils and waters contaminated with toxic metals. *Emerging technologies in hazardous waste management VI*. ACS Industrial & Engineering Chemistry Division Special Symp Atlanta
41. Rahman A, Nahar N, Nawani NN, Jass J, Ghosh S, Olsson B, Mandal A (2015) Comparative genome analysis of *Lysinibacillus* B1-CDA, a bacterium that accumulates arsenics. *Genomics* 106(6):384–392
42. Rotkittikhu P, Kruatrachue M, Chaiyarat R, Ngernsarsaruay C, Pokethitiyook P, Paijitprapaporn A, Baker AJM (2006) Uptake and accumulation of lead by plants from the Bo Ngam lead mine area in Thailand. *Environ Pollut* 144(2):681–688
43. Sardrood BP, Goltapeh EM, Varma A (2013) *An introduction to bioremediation. Fungi as Bioremediators*. Springer, Berlin, Heidelberg, pp 3–27
44. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc*

45. Schriefer AE, Cliften PF, Hibberd MC, Sawyer C, Brown-Kennerly V, Burcea L, Klotz E, Crosby SD, Gordon JI, Head RD (2018) A multi-amplicon 16S rRNA sequencing and analysis method for improved taxonomic profiling of bacterial communities. *J Microbiol Methods* 154:6–13
46. Schwitzguébel J (2000) Potential of phytoremediation, an emerging green technology. In: ecosystem service and sustainable watershed management in north China. Proceedings of International Conference, Beijing, P.R. China
47. Shen Z, Li X, Wang C, Chen H, Chua H (2002) Lead phytoextraction from contaminated soil with high-biomass plant species. *J Environ Qual* 31(6):1893–1900
48. Siddiquee S, Rovina K, Azad SA, Naher L, Suryani S, Chaikaew P (2015) Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: A review. *J Microb Biochem Technol* 7(6):384–395
49. Sisay B, Debebe E, Meresa A, Abera T (2019) Analysis of cadmium and lead using atomic absorption spectrophotometer in roadside soils of Jimma town. *J Anal Pharm* 8(4):144–147
50. Sriram MI, Kalishwaralal K, Deepak V, Gracerosepate R, Srisakthi K, Sangiliyandi Gurunathan S (2011) Biofilm inhibition and antimicrobial action of lipopeptide biosurfactant produced by heavy metal tolerant strain *Bacillus cereus* NK1. <https://doi.org/10.1016/j.colsurfb.2011.02.026>
51. Tariq FS, Samsuri AW, Karam DS, Aris AZ (2016) Phytoremediation of gold mine tailings amended with iron-coated and uncoated rice husk ash by vetiver grass (*Vetiveria zizanioides* Linn.). *Appl Environ Soil Sci*. <https://doi.org/10.1155/2016/4151898>
52. United States Environmental Protection Agency (2001) A citizen's guide to bioremediation. <https://www.epa.gov/remedytech>
53. Vidali M (2001) Bioremediation. An overview. *Pure Appl Chem* 73(7):1163–1172
54. Vijayaraghavan K, Yun YS (2008) Bacterial biosorbents and biosorption. *Biotechnol Adv* 26(3):266–291. doi: 10.1016/j.biotechadv.2008.02.002
55. Watanabe T (2010) Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key species. Third Edition. CRC Press. Taylor and Francis Group. 397
56. White C, Gadd G (1986) Uptake and cellular distribution of copper, cobalt and cadmium in strains of *Saccharomyces cerevisiae* cultured on elevated concentration of these metals. *FEMS Microbiol Rev* 38:227–283
57. Yan A, Wang Y, Tan SN, Yusof MLM (2020) Subhadip Ghosh and Zhong Chen Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. <https://doi.org/10.3389/fpls.2020.00359>. *Front Plant Sci*
58. Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci Total Environ* 386(2–3):456–464
59. Zhang MK, Liu ZY, Wang H (2010) Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. *Commun Soil Sci Plant Anal* 417:820–831

Tables

Table 1. Percent occurrence of different fungal isolates grown on PDA acquired from soil samples contaminated with mine tailings

Species of fungal isolates	Percent occurrence
<i>Penicillium janthinellum</i>	30.56
<i>Trichoderma hamatum</i>	52.78
<i>Trichoderma harzianum</i>	11.11
<i>Curvularia lunata</i>	5.56

Table 2. Bacterial species isolated from mine tailings contaminated soil

Species of bacterial isolates	Identity	Accession number
<i>Bacillus cereus</i>	99.38%	MG206040
<i>Bacillus cereus</i>	100.00%	MK894129
<i>Bacillus cereus</i>	97.27%	JN206614
<i>Bacillus cereus</i>	99.34%	GU369810
<i>Bacillus cereus</i>	97.53%	MK346118
<i>Bacillus thuringiensis</i>	98.11%	MK277453
<i>Bacillus thuringiensis</i>	96.03%	FJ393313
<i>Psuedomonas gessardii</i>	99.40%	KC903222
<i>Lysinibacillus xylanilyticus</i>	100.00%	MK388392
<i>Lysinibasillus sphaericus</i>	100.00%	JF819704
Unidentified bacterium 1	na*	na*
Unidentified bacterium 2	na*	na*

* not applicable

Table 3. Amount of cadmium and lead in shoot and root parts of different plant species sampled in contaminated rice paddy soils

SAMPLES	CADMIUM (mg kg ⁻¹)		LEAD (mg kg ⁻¹)	
	Shoots	Roots	Shoots	Roots
<i>Cyperus difformis</i>	0.60	0.00	0.00	0.00
<i>Scirpus juncooides</i>	0.40	0.40	0.00	15.00
<i>Fimbristylis miliacea</i>	0.60	3.00	0.00	15.00
<i>Centella asiatica</i>	1.40	3.60	2.80	0.00
<i>Sphagneticola trilobata</i>	3.10	2.00	0.00	0.00
<i>Monochoria vaginalis</i>	0.00	1.80	0.00	5.60
Soil	0.50		17.00	

Figures

Figure 1

Collection site in Sitio Camalca, Brgy. Buhawen, San Marcelino, Province of Zambales, Philippines.
Sourced from Google Earth.



Figure 2

Bacterial colonies on PDA at different dilutions, (A) 10^{-1} ; (B) 10^{-2} ; (C) 10^{-3} ; (D) 10^{-4} ; and (E) 10^{-5}

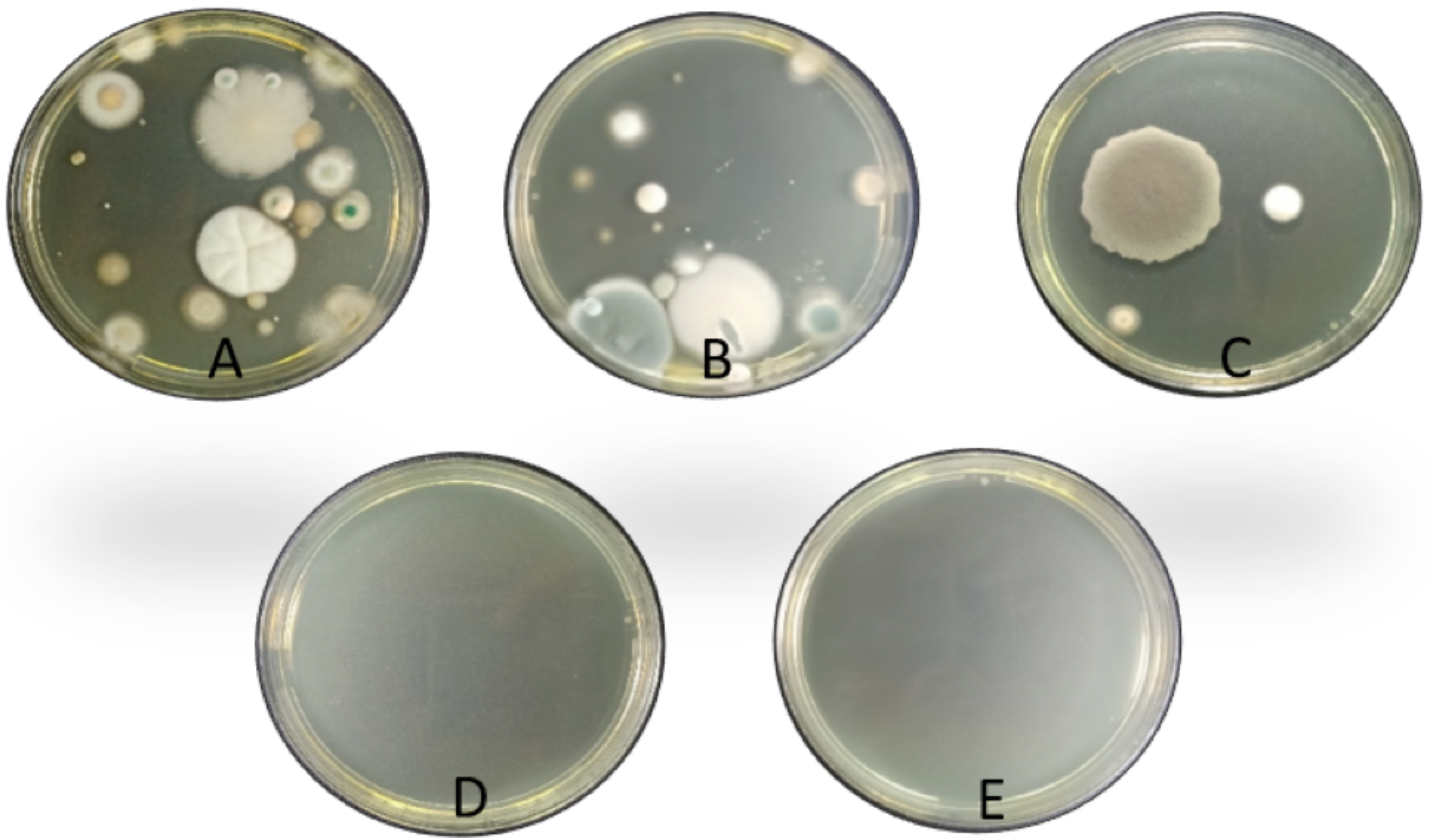


Figure 3

Fungal colonies on PDA at different dilutions, (A) 10^{-1} ; (B) 10^{-2} ; (C) 10^{-3} ; (D) 10^{-4} ; and (E) 10^{-5}

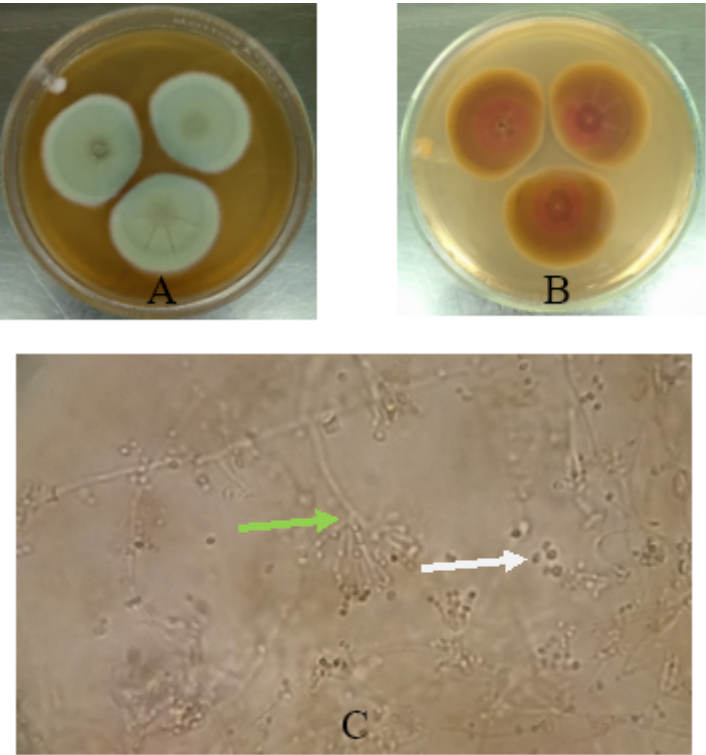


Figure 4

Cultural and morphological characteristics of *P. janthinellum* grown on PDA, 5 DAI at room temperature: (A) Obverse growth; (B) Reverse growth; and (C) Conidiophores (white arrow) and phialides (green arrow)

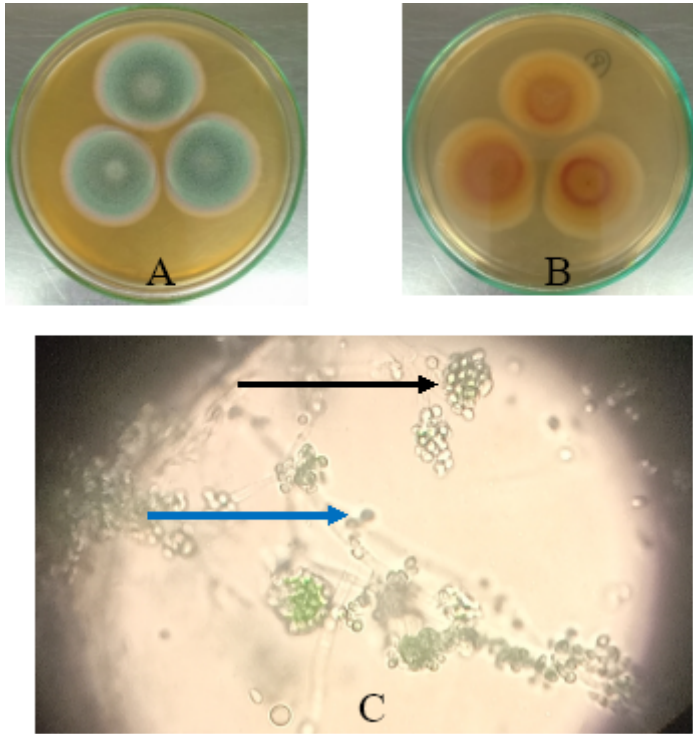


Figure 5

Cultural and morphological characteristics of *T. hamatum* grown on PDA, 5 DAI at room temperature: (A) Obverse growth; (B) Reverse growth; and (C) Conidiophores (blue arrow) and phialides (black arrow)

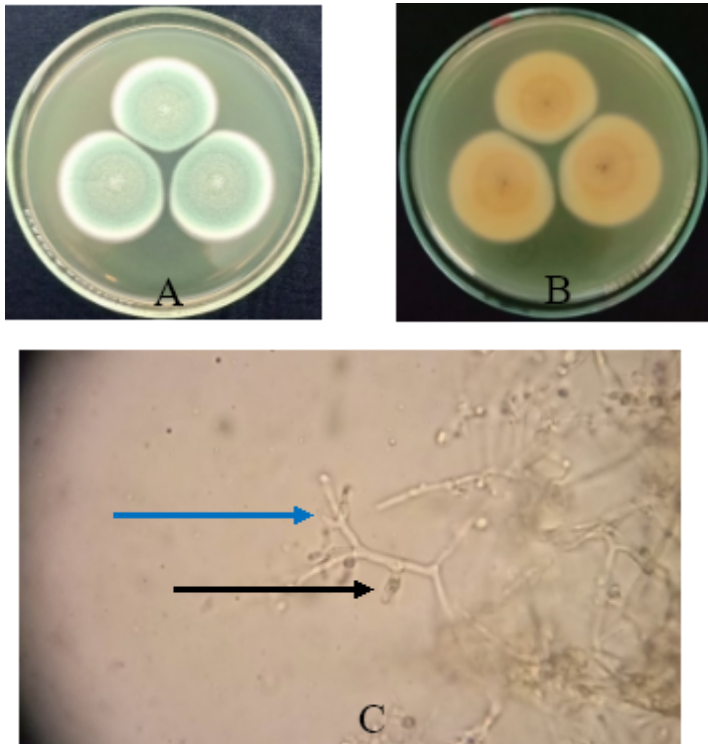


Figure 6

Cultural and morphological characteristics of *T. harzianum* strain with 99.99% identity (ACCC32889) grown on PDA, 7 DAI at room temperature: (a) Obverse growth; and (b) Reverse growth; and (c) Phialides (blue arrow) and phialospores (black arrows).

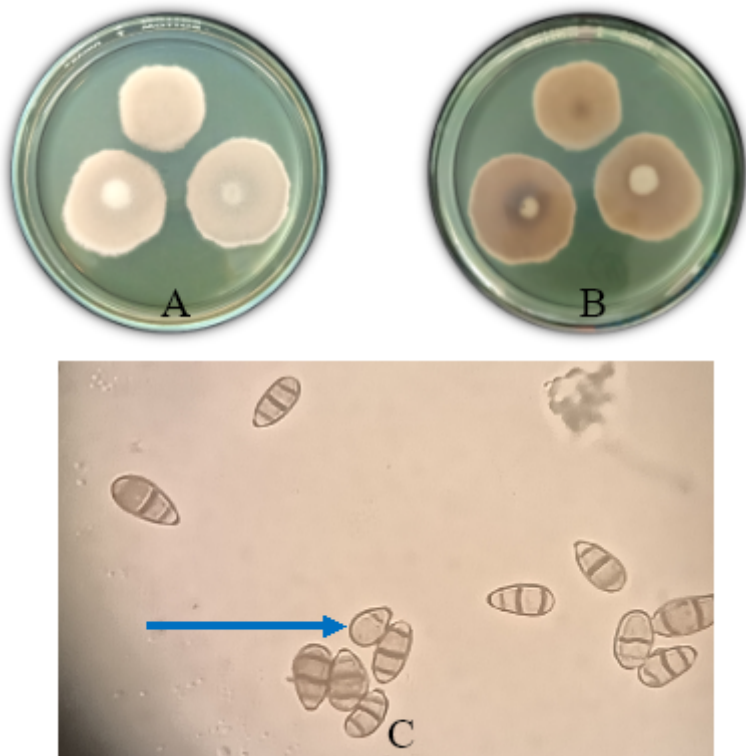


Figure 7

Cultural and morphological characteristics of *C. lunata* grown on PDA, 5 DAI at room temperature: (a) Obverse growth; (b) Reverse growth; and (c) Conidia (blue arrow)

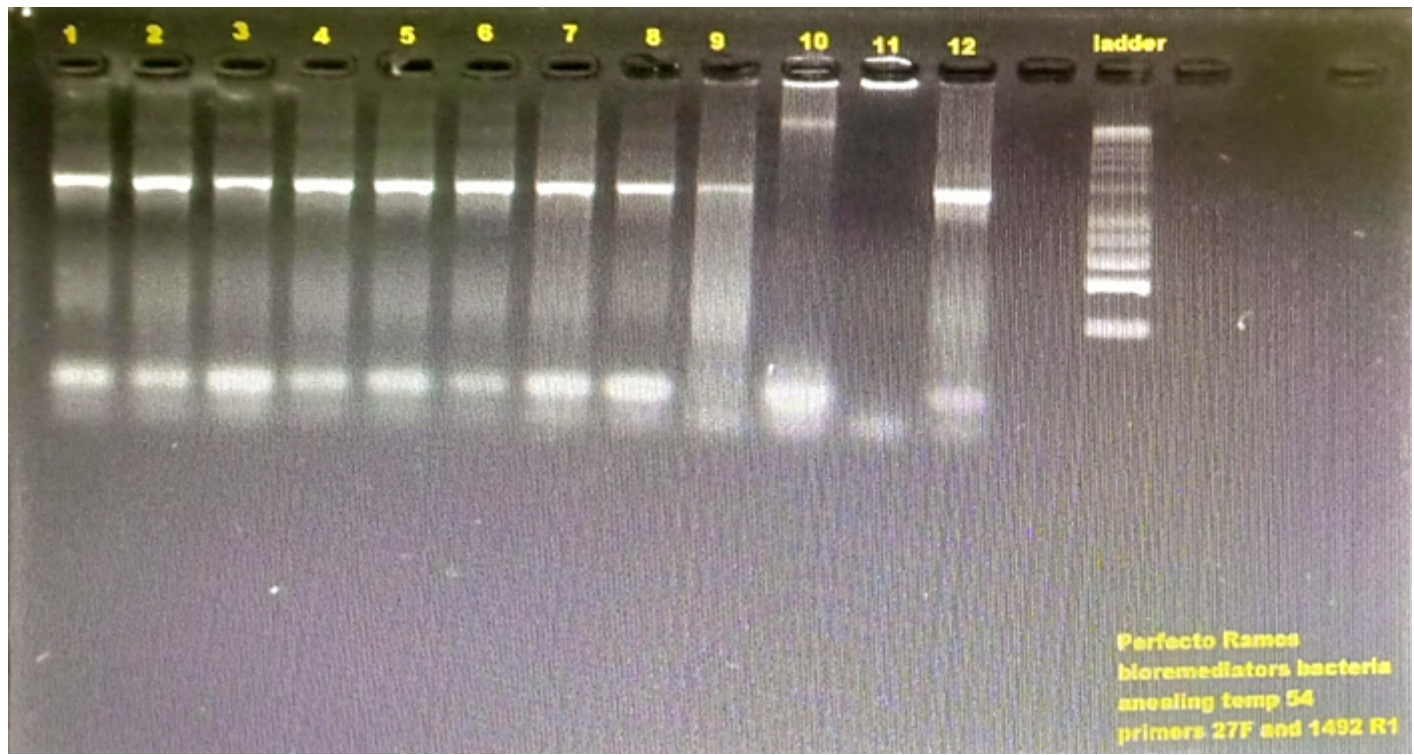


Figure 8

DNA band sequences of extracted bacteria on agarose electrophoresis. Lanes 10 and 11 are unidentified owing to absence of bands



Figure 9

Collected plant species from soil contaminated with mine tailings: (A) *Cyperus difformis*; (B) *Scirpus juncooides*; (C) *Fimbristylis miliacea*; (D) *Centella asiatica*; (E) *Sphagneticola trilobata*; and (F) *Monochoria vaginalis*